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The Agricultural Chemical Society of Japan.

President : Keijiro Aso.

The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2, No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japanese texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

STUDIES ON BAC. SAPROGENES SAKÉ. II.

By

Izue Yamazaki

(Received March 19th., 1929)

Bac. saprogenes Saké-Takahashi represents groups of bacteria which attack Saké and alter its value by the production of lactic acid and above all of an especially disgusting odor which is called "Hiochi" odor. (Klöcker, Gärungs-organismen, 1924).

T. Takahashi and his co-workers studied extensively (1906-1915) on 54 strains of this group, but the most important questions remained unsettled.

They are as follows:

(1) This group of bacteria can not develop in ordinary artificial media such as sugar-bouillon and Koji extract. In yeast water most of them grow but weakly.

Why so?

(2) In Saké, their natural habitat, also in pure culture, their growth is generally weak; and they easily degenerate within several successive transplantations.

Is there any most suitable medium?

Author discovered after long survey that liver-Saké medium, which is prepared by merely adding 2 or 3 autoclaved liver pieces to Saké following the same technic as in the preparation of the well known liver-bouillon medium, permited them to grow very luxuriantly.

In this medium they thrive without any degeneration for several years if transplanted once in 2 or 3 months.

Generally, liver-Saké medium serve them as an enrichment, regenerating, and stock media. And the starting obstacle being thus removed, it was possible to study this problem freely.

The author isolated 88 strains in pure culture and 6 in sub-culture from samples of Saké gathered from several districts in Japan, and studied their bacteriological and biochemical features.

Cultural and morphological characters are issued in this report.

1. They are all Gram positive.
3. They are all non-motile and sporeless.
3. According to their cultural characters in the liquid media, they are classified in 4 groups as follows:

T. Takahashi, Bullet. of the College of Agricult. Tokyo Imp. Univ. Japan. Vol. VII, No. 4, 1907.

group	No. of strains	media used			
		Koji extract	dextrose-bouillon		dextrose yeast water pH 4.3
			neutral	acid pH 4.3	
A. group. A.F. 1 type	64	no growth	no growth	no growth	moderate growth
A.F. 100 type	18	no growth	no growth	weak growth	difficult or no growth
B. group.	4	good growth	no growth	no growth	moderate growth
C. group.	8	good growth	good growth	good growth	good growth

4. By the liver-Saké agar stab culture, they are also divided in 2 groups:
   a) Good growth in the canal, not on the surface.
      A. group, A F. 1. type .....64 strains
      B. group, ... 4 〃
      C. group, ... 8 〃
   b) No growth in the stab culture.
      A. group, A.F. 100. type .....17 〃
5. Cell form and its arrangement: They are all slender rod, the length varies according to the medium, but the width remains constant.
   In the liver-Saké culture;
   0.5—0.6μ × 2—4—6μ, single or 2—3 in chain
   In the yeast water culture;
   generally cell elongates and curls, 0.5—0.6μ × 4—10—20—40μ.
   In the Saké-agar stab culture;
   generally cell shortens but arranges in endless chain, 0.5—0.6μ × 1.5—2.0μ.

STUDIES ON BAC. SAPROGENES SAKÉ. III.

GROWTH OF THE BACILLI AND ITS ACCESSORY FACTOR.

By

IZUE YAMAZAKI

(Received March 19th., 1929)

Bac. saprogenes Saké can grow in Saké but not in the usual artificial media, such as bouillon or Koji extract.

Regarding this peculiar fact, it has long been assumed that some intimate relation should exist between some constituents of Saké and the growth of the bacilli.

Recently K. Kurono proposed a vitamin like substance, absorbable by active carbon, in Saké which is neccessary for the development of the bacilli.

The author studied on this problem and reached the following conclusions:

(1) Acid (pH. 4.3)- dextrose-bouillon when mixed with a little Saké (2—10% by vol.) permits good growth for the bacilli.

(2) This bouillon activating factor cannot be absorbed out of Saké by active carbon, and also cannot be precipitated by any of the following reagents: that is, alcohol plus ether; basic lead acetate; H_2SO_4-phosphotungstic acid; Neuberg's soda-mercuric acetate; and $CuSO_4$-milk-of-lime carbohydrate precipitant.

(3) But it can be extracted by ether, in acid reaction. 0.05—0.1c.c. of this acidic extract can activate 10c.c. bouillon as well as Saké itself.

(4) Ether extract of long autolysed (2—6 months) yeast solution in strong acidic condition also contains this factor, 0.05—0.1c.c. of this acid extract activates 10c.c. bouillon as well as Saké extract.

(5) Thus one can cultivate the bacilli in acid-dextrose-bouillon plus yeast extract entirely free from Saké.

(6) Upon yeast water and Koji extract media, this factor is of no effect.

(7) Koji extract itself exerts much the same activating effect as Saké when added to bouillon.

(8) In Koji-extract medium itself, when it is prepared in fresh and unheated condition, most of the bacilli grow as normally and abundantly as in bouillon plus Saké medium.

(9) The above results were all well verified experimently with several decades of strains among the author's collection.

(10) The nature of the factor which controls the growth of the bacilli cannot be ascertained to be the same as that of vitamins in the animal region.

One can conclude with much certainty that the factor affects the physico-chemical character of the medium so as to afford the suitable environment.

STUDIES ON BAC. SAPROGENES SAKÉ. IV.

STRICT ANAEROBIC CULTURE IN ACID SIDE (pH 3.6—6.0). CYSTEIN-AGAR-SEAL-METHOD.

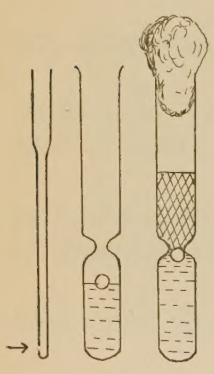
By

IZUE YAMAZAKI

(Received March 19th., 1929)

Saprogenes Saké groups of bacteria are all lactic acid formers and strongly aciduric, and alcoholphylic, so to speak. Their optimum pH range for growth lies between 4.0 and 5.0. It is important to determine the attitude of lactic acid bacteria towards strict anaerobic condition. But in acid media whose pH is 5.0 or less, any of the existing methods is of no use.

By the combination of Hosoya's cystein method and Kadisch's agar seal method, the author succeeded in this attempt. The autoxidising power of cystein decreases markedly along with the increase of H–ion concentration, but if it were protected entirely from air oxygen, cystein consumes all free oxygen in media and reduces methylenblue indicator to leuco–base and thus creates strict anaerobic condition.



Apparatus consists in an anaerobic tube, which is the modification of Kadisch tube by the author, and has a small empty glass ball which floats on the surface of the medium and acts as a valve when full filled. This glass ball in one way prevents, as valve, melted agar when poured in for sealing, from creeping down, and in the other, acts as a stirer in the medium by turning the tube up and down.

Procedure:

1. Fill the anaerobic tubes with bouillon, yeast water or Koji extract of a certain pH, up to the narrow neck, plug with cotton, and sterilize in an autoclave.

2. Weigh out cysteine– HCl 0.5 to 2.0mg. each per 10c.c. of medium, dissolve in a little water, add one drop of phenol red indicator, if neccessary, and sterilize as above.

3. Distribute the sterile cysteine–HCl solution by a capillary pipette to each sterilized medium, if reagent is neutralised to phenol red just before distribution, there is absolutely no fear of any change in pH value of medium.

4. Fill with sterilized water, if neccessary, up to the neck.

5. Pour into the tube some sterilized agar which is melted and cooled

to about 42—45°C., to the height of ab. 3cm., plug with cotton, leave to cool.

These procedures (3—5) are carried out in a sterile chamber.

6. After agar is hardened, invert the tube and stir up the inner part of medium, incubate at ab. 30°C.

7. After 10 to 24 hours, medium becomes strictly anaerobic, which condition may be clearly recognized by the decolorization of the methylenblue of the control tube.

8. Inoculate with bacteria by thrusting capillary pipette through agar seal and inject about one drop volume of bacterial suspension. If we use a capillary pipette which has an opening on side wall just above the fused head, there is no fear of clogging with agar pieces. And the inoculating canal thus made through agar is quite air-tight. Stir well by turning the tube up and down, incubate at a suitable temperature.

Experiment 1. pH. effect on the growth in the aerobic and the anaerobic condition.

Medium .....dextrose-bouillon mixed with Saké (10%).
Strain .....A.F. 101.
Inoculation.....0 03c.c. from the liver-Saké culture, 10 days old.

after 10 days incubation at 30°C.	pH. 4.0	pH. 4.3	pH. 5.0	pH. 6.0	pH. 7.0
cystein-agar seal anaerobic culture	++ . +++	++ . +++	++ . +++	− . ±	—
aerobic culture	− −	− ±	− ±	—	—

Notice.		strong.	moderate.	weak.	non.
Growth	Turbidity	++	++	+	−
	Sediment	+++	+++	+	−

After 30 days incubation, pH and titratable acidity are determined :

pH		Titration Acidity per 10c.c. with 1/10 N. NaOH.		Acid produced as lactic %
initial	last	initial	last	
4.0	3.4	4.8c.c.	14.8c.c.	0.90
4.3	3.6	3.2	11.6	0.76
5.0	3.6	2.4	11.6	0.83
6.0	3.7	1.8	11.6	0.88

Experiment 2.

Medium. .....dextrose (0.5%)-acid (pH. 4.5)-bouillon mixed with Saké (10%).

According to the anaerobic culture by the cystein-agar seal method, 85 strains of the Bac. saprogenes Saké are divided in 2 groups.

I. Group. .....Good growth in strict anaerobic condition.....18 strains.
II. Group. .....Very faint growth " " .....67 "

Literature.

S. Hosoya: The Scientific Report f. the Governm. Inst. f. Infect. Dis. Vol. IV. 1925.
E. Kadisch: Centralbl. f. Bakt. Abt. I. Orig. B. 91, 1923—24.

STUDIES ON THE GERMINATION OF SEEDS. PART II.

STARCH PRODUCED DURING THE GERMINATION OF SOY-BEAN SEEDS.

By

SHUIKU SASAKI.

(From the Biochemical Laboratory, Department of Agriculture, Kyushu Imperial University.)

(Received April 23rd., 1929)

Many investigators have studied starch in soy-bean seeds ; some authors found several per cent of starch, while others found practically none. These discrepancies are probably due to the stage of ripening of the beans, or to the way in which they are allowed to ripen. It is certain that starch content decreases gradually with the ripening process and, finally, at the stage of complete ripening, it is so little as to scarcely be capable of bring detected.

During our studies of the germination of seeds, we found that when soy-beans are germinated in the dark much starch is produced in the seedlings.

A. *Detection and isolation of starch in seedlings.*

The section of the cotyledon, two or three days after sowing, is coloured blue by a drop of iodine solution and shows many little starch granules under the microscope. These cannot be detected in the original soy-bean seeds before germination.

For the purpose of isolating the starch, we used the following method. Seedlings are well washed, ground, mixed with 5 % NaCl solution and filtered off by a Buchner's funnel through a thin layer of filter paper pulp placed on the usual quantitative filter paper. As the sizes of starch granules are very little they pass easily through the filter with NaCl mixed solution. The residue on the filter is again ground, mixed with NaCl solution and filtered off. This process is repeated till all the starch granules have passed completely through the filter. The starch and NaCl mixed solution is separated by a centrifugal machine. The precipitate is agitated with NaCl solution and again centrifuged, and then extracted with 0.2% NaOH solution to solve protein and other impurities. The residue from NaOH solution is treated with distilled water, alcohol and ether successively. This product is free from protein, cellulose and other impurities, and consists of lmost pure

starch.

The quantity of starch in seedlings gained by this method is shown in the following table, in per cent of the original weight of soy-bean seeds.

Name of species \ Days after sowing	1	2	3	4	5	6	7	8	9
Ôshoku akidaizu, % (a kind of autumn seed)	0.4	1.7	3.4	4.4	5.1	4.8	5.0	4.7	4.8
Taishu natsudaizu, % (a kind of summer seed)	0.5	2.8	—	5.8	—	7.5	—	7.7	—

This difference of figures for the two species is due rather to the difference in variety of seeds, than to the experimental error. The original soy-bean seeds contain practically no starch, and moreover their solution is so viscous that filtration and centrifuging are very difficult, and isolation of starch has not been successfully accomplished.

B. *Characters of starch.*

The starch of seedlings of soy-bean was compared with several other typical starches in physical and chemical characters. Microscopically these starch granules are circular or nearly so, and neither hilum nor eccentric rings is visible in the majority. A cross appears when examined by polarised light. The sizes are so small that they make Brownian movement in water, having diameter $1/2\mu$ to 4μ. Except for its size, the microscopical characters of this starch are analogous to those of the starch of wheat and of the leguminous groups. The gelatinisation-temperature is $70° \pm 2°$C. On hydrolysis with dilute hydrochloric acid it converts completely into dextrose. After liquifying by boiling water under atmospheric pressure and under higher pressure, the saccharification velocities of this starch by Taka-diastase are slower than that of potato starch and faster than that of wheat starch.

ON THE PHYTASE OF ASPERGILLUS SPECIES.

By

Tsunejiro Kawahara.

(Received April 25th., 1929)

Since Suzuki, Yoshimura and Takaishi reported the existence of phytase in rice bran many other investigators have studied on this enzyme. Especially in the field of microbes only such workers as Dox and Golden, Jegorow

and C. Shimoda have published the result of their researches on the phytase. The author examined the distribution of phytase in Aspergillus species, and investigated its natures. The results were summarized as follows :–

1. Asp. oryzae A, Asp. oryzae C, Asp. oryzae E, Asp. oryzae F, Asp. oryzae L, Asp. oryzae M, Asp. oryzae N, Asp. niger, Asp. melleus, Asp. varians, Asp. giganteus and Asp. parasiticus which were used in the experiments, all have the phytase and always the intra-cellular enzyme was more active than the extra-cellular's.

2. The optimum temperature of activity for the phytase was 50°C and the optimum hydrogen ion concentration of this enzymic action was 4.67. The later was not influenced by the difference of the buffer solutions, but the citrate mixtures of Sörensen gave the worse influence to the activity of phytase than the Valpole's acetate mixtures.

3. As the spliting products of phytin by the action of phytase were isolated inosite, inosite monophosphate, dibarium inosite triphosphate and neutral barium salt of inosite triphosphate. The analytical results were as follows :

	C%	H%	P%	Ba%
(a) Found	27.43	5.35	11.75	—
Calculated for inosite monophosphate $C_6H_{13}O_9P=260$	27.69	5.00	11.92	—
(b) Found	39.82	6.97	—	—
Calculated for inosite $C_6H_{12}O_6=180$	40.00	6.66	—	—
(c) Found	11.57	2.08	12.67	39.85
Calculated for dibarium inosite triphosphate $C_6H_{11}O_{15}P_3Ba_2=690$	10.46	1.59	13.47	39.71
(d) Found	9.50	1.58	11.03	48.26
Calculated for neutral barium salt of inosite triphosphate $C_6H_9O_{15}P_3Ba_3=826$	8.71	1.08	11.25	49.88

In the results of (c) and (d), the percentages of carbon were high and that of phosphorus were low. It was supposed that the preparates were somewhat impure and mixed the barium inosite diphosphate, but the lack of sample has prevented the further experiments.

From these results, it was known that the action of phytase upon phytin seems to proceed in several stages.

STUDIES ON THE CASTOR-BEAN LIPASE. III.

By

Etsuo Takamiya.

(Received May 8th, 1929)

I. *Influence of Ultra-violet Rays upon the Enzyme.*

Two enzyme preparations, of which one is natural and the other highly purified, were irradiated through a quartz and a ultra-filter plate in a water-jacketed box, regulating temperature during irradiation. The experiments showed that irradiation with a mercury vapour quartz lamp have no influences at all upon the enzymic activity.

II. *Enzymic Hydrolysis of Vegetable Oils, and Vegetable Oils Irradiated with Ultra-violet Rays.*

Six vegetable oils, differing in iodine value—coconut oil, olive oil, almond oil, cottonseed oil, soy-bean oil, linseed oil—were employed in this study.

There exists a certain correlation between the iodine value of oils and their hydrolysis-velocity by the enzyme; namely, the greater the iodine value the less is the velocity. From this fact, obtained as the results of experiments, we can assume that the nutritive value of fats and oils, which is proportional to the velocity of hydrolysis, is inversely proportional to their iodine values. This assumption will also be confirmed by the results of feeding experiments which were carried out by other investigators such as J. Ozaki and S. Ueno.

By the irradiation with ultra-violet rays, it resulted that the vegetable oils were less readily hydrolysed by the enzyme than were the original oils and the greater the iodine value, the greater was the influence of the irradiation; and that the iodine values of the irradiated oils become less than those of the original, and the greater the iodine value the greater is the influence of the irradiation; and also that the decrease in degree of the enzymic hydrolysis-velocity of oils was far greater than that of the iodine values. The decrease caused by the irradiation in the enzymic hydrolysis-velocity of oils will be concluded, therefore, to be more probablly due to the fact that the presence of oils altered by the irradiation retards in some way the enzymic activity, rather than to the fact that such altered oils are no longer hydrolysed by the enzyme.

STUDIES ON THE FERMENTATION PRODUCTS BY MOULD FUNGI. IV.

ASPERGILLUS GLAUCUS. PART I.

By

YUSUKE SUMIKI.

(Agricultural Chemical Laboratory, Tokyo Imperial University)

(Received May 13th., 1929)

I. Condition of the Cultivation.

Aspergillus glaucus was cultured at 30°C during 10—180 days in the following medium.

C-source : glucose or saccharose, 10%.
N-Source : Na-nitrate, -nitrite, Am.-nitrate, -sulphate, peptone, urea, asparagine, aspartic acid, glutamic acid, glycocoll, leucine, tyrosine, alanine, etc. 0.08—2.0%
Mineral matter : K-monophosphate & -diphosphate, each 0.015%.
Mg-sulphate & Ca-chloride, each 0.010%.
Fe-chloride & Na-chloride, each trace.
Ca-carbonate, if necessary.

II. Isolation of the Fermentation Products.

(a) The fermented medium is filtered, added with sulphuric acid and distillated with steem to isolate volatile substances from nonvolatile substances. The residue is evapolated to a small quantity and extracted with ether. After removal of ether, the crystal is dried on the tile and its melting point was examined (the m. p. of mixture with pure substance is also observed). When the crystals are the mixtures of 2 or more acids, they are isolated by adding Ca-acetate and Ca-hydroxide (see Bull. Agri. Chem. Soc. Japan., **2**, 94, 1926).

(b) The fermented medium is evapolated, neutrallized with ammonia and added with Pb-acetate. Ca-gluconate and -lactate remain in the filtrate. The filtered precipitate is treated with dil. acetic acid at 70°. Pb-malate goes into solution. The in acetic acid insoluble part of Pb-ppt. is treated with SH_2. After evapolation of this filtrate Ca-oxalate is precipitated by addition of acetic acid and Ca-chloride. After 24 hours the ppt. is filtered and from the filtrate tartaric acid is precipitated as wein stone adding K-acetate and 2 volumes of alcohol. After addition of ferric chloride and removal of Fe-

succinate, the filtrate is acidified, extracted with ether and then from evapolated solution citric acid is precipitated as Ba-citrate by adding Ba-acetate and 3 volumes of alcohol.

III. Identification of the Fermented Products.

1. Ethyl alcohol and volatile acid.

Volatile acid is not produced, but nearlly trace of ethyl alcohol is produced, for the distilate of the steem distillation shows neutral reaction with litmus paper and gives jodeform reaction after 24 hours but does not change the colour of Schiff's reagent.

2. Fumaric acid.

M. p. : 282—3° (sealed capillary, recrystallized from hot water).

Ag-salt. Subst. 0.5856 g. AgCl 0.5020 g.
$C_4H_2O_4Ag_2$ Cal. Ag 65.40%.
Fou. Ag 64.46%.

3. Succinic acid.

M. p. : 183—4° (recrystallized from hot water).

Ag-salt. Subst. 0.1024 g. AgCl 0.0878 g.
$C_4H_4O_4Ag_2$ Cal. Ag 65.06%.
Fou. Ag 64.54%.

4. Citric acid.

M. p. : 153—4° (recrystallized from hot water and dried in the vacuum decicater over sulphuric acid).

Ag-salt. Subst. 0.5996 g. AgCl 0.5010 g.
$C_6H_5O_7Ag_3$ Cal. Ag 63.12%.
Fou. Ag 62.89%

5. Oxalic acid.

Hydrous crystal melts at 100° and the m. p. of the mixture with pure hydrous oxalic acid shows no depression. The colour reaction by ferric chloride is positive.

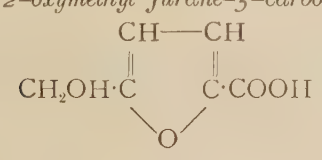
6. Tartaric acid.

Anhydrous crystal melts at 168° and the m. p. of mixture with pure tartaric acid shows no depression. It gives the reaction of resorcin-H_2SO_4 by heating.

7. Malic acid.

The melting points of the crystal and of the mixture with pure malic acid are both 100°. It gives diazo-reaction without heating.

8. 2-oxymethyl furane-5-carbonic acid.



By the method of isolation (a), the crystal which melts at 150—8° is obtained in some cultures. Recrystallizing from boiling water it crystallizes in long plate or

prism and melts at 163—4° (incorr.). It contains no nitrogen.

The elementary analysis is as follows :

1.	Subst. 0.1402 g.	CO_2	0.2604 g.	H_2O	0.0534 g.
2.	" 0.1398 g.	"	0.2604 g.	"	0.0564 g.
1.	C% = 50.66,	H% =	4.23.		
2.	" 50.80,	"	4.48.		
aver.	" 50.73,	"	4.36,	O% =	44.91.

Thus, C : H : O = 1.5 : 1.5 : 1. ∴ Empirical formula = $C_3H_3O_2$.

Determination of the molecular weight is as follows :

1. 0.0584g Subst. requires to neutralise 3.90c.c. of ca. N/10 KOH, i.e. 3.8965 c.c. of N/10 KOH (indicator : phenolphthalein). ∴ Mol. weight. = 149.
2. Subst. 0.0128g. Camphor 0.1274g. ΔT = 28° ∴ M. w. = 144.
3. " 0.0158g. " 0.1512g. " = 27° " 144.

From the above results the molecular formula for this substance is given as $C_6H_6O_4$.

The existence of "furane nucleus" is proved by the colour reaction of isatin–H_2SO_4(violet colour).

The existence of double bond is also proved by absorption of bromine and decoloriasation of K–permanganate.

Moreover, the melting point of the mixture with pure 2–oxymethyl furane-5–carbonic acid (m. p. 163—4°) which I synthesized (Fenton & Robinson : Jour. Chem. Soc., **95**, 1339, 1909 ; Fenton & Gostling : Jour. Chem. Soc., **75**, 429, 1899) shows no depression. Therefore it is an established fact that the crystal melting at 163—4° is 2–oxymethyl furane–5–carbonic acid.

9. An unknown substance.

It is a colourless crystal melting at 238—9° (incorr.) which contains no nitrogen. It is soluble in ether, alcohol but insoluble in water or boiling water. It is easily purified by dissolving in boiling water and then in alkali and precipitating by acid. I promise here that there will be a chance to write its constitution another day.

IV. Estimation of the Fermented Product.

The medium used is as follows :

1.	Am.–sulphate 0.08%.	3.	Peptone 0.08%.	5.	Na–nitrate 0.08%.
2.	" 2.00%.	4.	" 2.00%.	6.	" 2.00%.

The amounts of acids which were produced was titrated with ca. N/10 NaOH, using phenolphthalein as indicator and showed in the following table as c.c. of ca. N/10 NaOH in 100 c.c. of medium.

The amount of glucose which remained in the medium was estimated by Bertrand's method and showed as g. in 100 c.c. of medium in the following table.

	days.	4	6	8	10	12	14	17	19	23	25	27	29
1	acid	2.5	3.0	3.5	4.5	5.5	4.5	5.5	7.0	7.0	7.6	8.0	10.5
	glucose	6.78	6.59	6.45	6.35	6.35	6.29	6.29	6.18	5.85	5.85	5.85	4.23
2	acid	6.5	7.0	9.0	9.5	9.5	10.0	10.0	14.0	14.0	10.5	10.5	10.5
	glucose	6.78	6.59	6.59	5.85	5.65	5.52	5.40	5.28	5.28	5.28	5.28	5.28
3	acid	0	0	2.0	4.0	7.5	15.0	17.5	18.0	22.0	25.0	25.0	30.5
	glucose	6.78	6.52	6.42	6.12	5.83	5.52	5.52	5.28	4.95	4.93	4.85	5.23
4	acid	0	0	15.0	39.5	28.0	28.0	14.5	0	0	—	—	—
	glucose	5.40	4.93	2.62	1.82	0.66	0.64	0.53	0.48	0.35	—	—	—
5	acid	0.25	0.5	0.5	0	0	2.0	48.0	38.0	23.0	—	—	—
	glucose	7.64	7.45	7.19	7.15	6.59	6.07	5.82	5.45	5.09	—	—	—
6	acid	0	0	0	0	0	3.2	3.8	8.0	14.4	—	—	—
	glucose	6.95	6.55	5.60	5.59	5.72	5.72	2.95	2.16	1.02	—	—	—

V. Pigment.

About 10 years ago, Mr. Klöcker found that this fungus produced the pigment similar to fluorescein (Centbl. f. Bakt., 2 Abt. **46**, 1918). I wished to isolate as a crystal this pigment but could not.

First of all, I tried to discover the best condition to produce this pigment and found amoung the source of nitrogen peptone or Na–nitrate is the best. The effects of metalic salts were also examined and it was fouud that Sn-chloride, Al–sulphate, Pb–nitrate, Mn–chloride and Cd–chloride (each 0.04%) are more or less better to produce this pigment.

This pigment is easily soluble in ether, metyl alchol, ethyl alcohol, aceton, chloroform but not easily in benzene, peteroleum ether and water. When the aqueous solution of this pigment is added with ammonia, fluorescence appears, but by addition of acid it disappears.

VI. Conclusion.

1. The fermentation products from glucose and saccharose by Aspergillus glaucus was studied.

2. Fumaric, citric, succinic, oxalic, tartaric and malic acids were isolated and identified.

3. Beside these acids, 2 new substances were isolated. And it was identified that one of them was 2–oxymethyl furane–5–carbonic acid.

4. Non–volatile acid was not produced but nearly trace of ethyl alcohol was produced.

5. The effects of nitogen source and metalic salts on the pigment similar to fluorescein were studied but this pigment was not isolated as a crystal.

I desire to express my best thankss to Prof. Takahashi and Prof. Yabuta for their continued criticism and advice.

STUDIES ON THE FERMENTATION PRODUCTS BY MOULD FUNGI. V.

DEMATIUM PULLULANS.

By

YUSUKE SUMIKI.

(Agricultural Chemical Laboratory, Tokyo Imperial University)

(Received May 23rd., 1929)

I. Cultivation.

Dematium pullulans, pure cultured, was inoculated in the following mediums and cultivated at 30°C for several weeks.

	glucose	peptone	$(NH_4)_2SO_4$	K_2HPO_4 and KH_2PO_4 each.	$MgSO_4$ and $CaCl_2$ each.	$FeCl_3$ and NaCl each.	$CaCO_3$	I.	days
(a)	7.2%	0.10%	——	0.015%	0.010%	trace	——	15	50
(b)	〃	0.50%	——	〃	〃	〃	1%	5.2	75
(c)	〃	0.08%	——	〃	〃	〃	——		5—31
(d)	〃	——	1.0%	〃	〃	〃	——		5—31
(e)	〃	1.0%	——	〃	〃	〃	——		5—31

II. Isolation and Identification of the Fermented Products.

The fermented mediums (a and b) are acidified with sulphuric acid and distillated with steem. The distillate is added with the excess of Ba-carbonate and boiled to neutralize the volatile acid under the reverted cooler. The filtrate, removed from $BaCO_3$ by filtration, is distilled again to isolate alcohol and aldehyde from the Ba-salt of volatile acid. The distillate is distilled fractionally for several times and a fraction, b. p. 78°, is obtained. By repeated fractional distillation, 2 fractions, one of them gives the reaction of aldehyde with Schiff's reagent and the other does not, are obtained. The former is added with the saturated solution of Na-bisulphite, extracted with ether, made to alkaline with Na-carbonate and distilled. Thus a fraction which contains acetaldehyde is obtained. The latter is added with the excess of Ca-oxide, filtered, distilled and dehydrated absolute ethyl alcohol is isolated.

The aq. solution of the Ba-salt of volatile acid is evapolated to dryness and the Ba-salt is recrystallized from hot water.

The residue of the steem distillation of fermented medium is evapolated to a small quantity and extracted with ether. From the ethereal solution, a syrup is obtained with the crystal of succinic acid. After the removal of this

crystal, the syrup is neutrallized with Ba–hydroxide and added with 5 volumes of ethyl alcohol (95%) to precipitate the Ba–salt of succinic acid only. Ba-succinate is filtered off and the filtrate is evapolated, added with sulphuric acid and extracted with ether. From this ethereal solution, pure lactic acid is obtained.

Succinic acid.

M. p.: 184°. Yield: 1.2 g. from (a), 1.8 g. from (b).
Ag–salt. Subst. 0.9756 g. AgCl 0.8344 g.
$C_4H_4O_4Ag_2$ Cal. Ag 65.06%
Fou. Ag 64.48%

Lactic acid.

$\alpha=0$. Yield: 2.4 g. from (a), 3.2 g. from (b).
Zn–salt. Subst. 0.8344 g. ZnO 0.1145 g.
$(C_3H_5O_3)_2Zn$ Cal. Zn 26.74%
Fou. Zn 26.14%

Acetic acid.

The Ba–salt of volatile acid is added with sulphuric acid and distilled. This distillate has no reducing power, so formic acid is not produced.

Ag–salt. Subst. 0.1032 g. AgCl 0.0874 g.
$C_2H_3O_2Ag$ Cal. Ag 64.64%
Fou. Ag 63.75%

Ethylalcohol.

A fraction, b. p. 78°, dehydrated by the addition of Ca–oxide, is treated with the theoretical quantity of phenyl isocyanate. Phenyl urethane, m. p. 52°, is obtained by recrystallizing from hot alcohol.

Subst. 0.0858 g. N_2 6.60 cm³. (32.5°, 759.7 mm.)
$C_9H_{11}O_2N$ Cal. N 8.49%
Fou. N 8.22%

Acetaldehyde.

The fraction isolated from ethyl alcohol by the method above described, yields hydrazide, m. p. 127°, on treatment with p–nitrophenylhydrazine.

Subst. 0.0368 g. N_2 8.20 cm³. (32.5°, 759.0 mm.)
$C_8H_9O_2N$ Cal. N 23.46%
Fou. N 23.79%

III. Quantitative Determination of the Fermented Products.

Ethyl alcohol is determined by the ordinally distillation method using picnometer and showed in the following table as wt. %.

Acids are determined by titrating with N/10 NaOH using phenolphthalein as indicator and showed in the following table as lactic acid (mg.) in 100 c.c. of fermented medium.

The amount of glucose remained in the medium, is determined by the

method of Bertrand and showed in the following table as g. in 100 c.c. of fermented medium.

days	acid			alcohol		glucose
	(c)	(d)	(e)	(d)	(e)	(c)
5	27	168	—	0.05	—	6.4
9	31	168	—	0.16	0.21	5.9
11	27	160	208	0.16	0.42	6.2
14	27	184	—	0.21	0.37	5.3
18	27	188	232	0.32	0.80	4.9
21	44	200	—	0.37	0.80	5.2
24	27	—	240	0.39	0.85	4.9
28	22	—	248	0.53	0.96	4.9
31	27	272	248	0.42	0.96	4.9

IV. Conclusion.

1. The fermentation products of glucose by Dematium pullulans were studied.

2. As the fermented products, ethylalcohol, acetaldehyde, acetic acid, succinic acid and lactic acid (optical inactive) were isolated and identified.

3. The amounts of the fermented products were very small and a greater part of glucose was not consumed. The maximum yields of ethyl alcohol and of acids from 7.2 g. of glucose were each 0.98 wt. % and 0.27 %.

ON THE CHANGES OF THE CONCENTRATION OF THE BLOOD CONSTITUENTS OF THE SILKWORMS FED WITH THE MULBERRY LEAVES AT THE DIFFERENT STAGES OF DEVELOPMENT.

By

Shigeru Nakajima.

(Received May 23 rd., 1929)

There has been published a large number of works,[1][2][3][4][5][6][7] on variations in the concentration of the blood constituents of the silkworms as related to such factors as sex, growth, and health conditions of the worms, but as far as we know, no work has been done on the influences of the different foods given to the worms on their blood constituents. The present work,

therefore, has been performed in order to throw some light upon this problem. In this experiment the constituents of the mulberry leaves at different stages of development were used.

Methods.

Sampling of the blood:– All our work on the determination of inorganic and nitrogenous constituents, have been done on the whole blood. The whole blood was drawn from the anale corn cut, and taken into a test tube dipped in ice and water. By this means precipitation, and coloring by the oxydase which preexisted in the blood, were effectively prevented.

The worms for experiments:– The worms which provided samples of blood were of a Chinese variety "Shinpaku" reared in the ordinary way until the fourth age. As soon as the fourth moulting was finished, the worms were separated in two groups according to sex, and the experiments were covered both sexes. The worms of each sex were divided into three groups, A, B and C. From the beginning to the end of the fifth age, group A was fed with the youngest leaves, group B with those in a medium stage of development, and group C with the full developed. Thus the three groups were reared in the same conditions except for food, and blood was taken every day from new individuals of the three groups. The constituents of the leaves for experiments are shown in the following table:

Table I.

	Water	Dry matter	Crude protein	Protein-nitrogen	Non-protein-nitrogen	Ether extract	Crude fiber	Crude ash	Carbo-hydrate
a	78.11	21.89	6.51	0.71	0.32	0.46	0.90	1.80	4.01
b	73.64	26.36	6.32	0.75	0.26	0.97	1.82	2.90	6.08
c	72.82	27.18	5.38	0.65	0.21	1.28	2.26	3.40	4.74

	SO_3	P_2O_5	Cl	Fe_2O_3	MgO	CaO	K_2O	Na_2O
a	0.09	0.27	0.01	0.18	0.12	0.34	0.56	0.12
b	0.08	0.18	0.01	0.16	0.24	0.64	0.58	0.14
c	0.07	0.14	0.02	0.14	0.24	0.76	0.44	0.13

Analytical procedures.

Determination of specific gravity:– Specific gravity has been calculated by weighing instantly after measuring 2 c.c. of the blood at 15° C by the Ostwald pipette.

Determination of hydrogen ion concentration:– Performed colorimetrically.

Determination of nitrogenous constituents:– Kjeldahl's method was applied for determining nitrogen, and non–protein nitrogen was determined in the filtrate of protein precipitated by trichloroacetic acid, while the protein nitrogen was calculated by reducing the non–protein–nitrogen from the total nitrogen.

Determination of the inorganic elements:– All the inorganic elements are determined in the trichloroacetic acid filtrate. Calcium is precipitated as oxalate after neutralizing the filtrate with dilute ammonia, using methyl red as an indicator as recommended by Shool.[8] The precipitate was filtered, washed, and titrated according to the technique of Simpsom[9] magnesium, potassium and the phosphate was determined by Briggs' modification of the Bell and Doisy method,[10] and chloride by the method of Whiterhon.[11]

Experimental results.

The daily results of the analysis of the blood of the three groups including two sexes are given in Table II.

TABLE II. (Female.)

Grams in 100 c c.

		Specific gravity	P_H	Water	Dry matter	Organic matter	Inorganic matter	Total nitrogen	Protein nitrogen	Nonprotein nitrogen
0		1.0222	6.1	96.78	5.44	4.806	0.636	0.366	0.231	0.153
2	A	1.0222	6.2	95.82	6.40	5.830	0.566	0.622	0.293	0.329
	B	1.0227	6.3	95.93	6.34	5.094	0.642	0.644	0.351	0.293
	C	1.0206	6.4	96.33	5.73	5.000	0.724	0.440	0.199	0.241
3	A	1.0262	6.3	94.77	7.85	7.124	0.724	——	——	——
	B	1.0267	6.3	94.98	7.69	6.970	0.722	——	——	——
	C	1.0236	6.4	95.86	6.50	5.836	0.664	——	——	——
4	A	1.0323	6.2	92.49	10.70	9.914	0.822	1.116	0 823	0.293
	B	1.0304	6.2	92.82	10.22	9.504	0.730	1.037	0.728	0.309
	C	1.0254	6.3	94.06	8.48	7.846	0.630	0.652	0.398	0.254
5	A	1.0318	6.4	91.78	11.40	10.652	0.748	——	——	——
	B	1.0347	6.4	91.47	12.00	11.264	0.736	——	——	——
	C	1.0272	6.4	93.41	9.31	8.676	0.638	——	——	——
When the worms began spinning	A	1.0435	6.3	91.29	13.06	12.308	0.756	1.282	1.021	0.261
	B	1.0390	6.4	90.85	13.05	12.294	0.756	1.260	1.019	0.241
	C	1.0364	6.3	91.21	12.43	11.692	0.740	1.104	0.839	0.265

Mg. in 100 c.c.

		Total phosphate	Organic phosphate	Inorganic phosphate	Chloride	Calsium	Magnesium	Potassium
0		47.9	41.3	6.6	29.0	51.3	149.4	227.0
2	A	85.3	75.4	9.9	29.1	54.6	132.2	215 0
	B	56.8	48.4	8.4	30.9	60.5	165.2	208.5
	C	47.9	40.7	8.2	34.5	68.1	172.4	213.0
3	A	110.2	——	——	——	52.2	145.0	——
	B	88.7	——	——	——	58.7	159.4	——
	C	54.5	——	——	——	66.8	169.4	——
4	A	146.1	135.2	10.9	32.7	50.6	146.6	191.5
	B	117.4	109.5	7.9	29.7	57.0	150.2	188.5
	C	69.4	63.0	6.4	31.5	62.5	160.4	196.5

5	A	141.1	——	——	——	48.8	141.8	——
	B	134.9	——	——	——	55.0	146.6	——
	C	92.6	——	——	——	57.7	163.0	——
When the worms began spinning	A	104.3	97.9	6.4	35.9	66.9	135.6	212.5
	B	90.1	84.1	6.0	31.7	68.9	165.0	199.5
	C	87.3	82.0	5.3	37.5	70.3	161.0	185.0

Male

Grams. in 100 c c.

		Specific gravity	P_H	Water	Dry matter	Organic matter	Inorganic matter	Total nitrogen	Protein nitrogen	Nonprotein nitrogen
0		1.0198	6.2	95.65	5.33	4.710	0.618	0.333	0.199	0.134
2	A	1 0246	6.3	96.62	5.84	5.260	0.575	0.532	0.223	0.309
	B	1.0228	6.4	96.69	5.59	4.980	0.608	0.498	0.201	0.297
	C	1.0210	6.4	96 63	5.47	4.75	0.720	0.378	0.160	0.218
3	A	1.0257	6.2	95.50	7.07	6.306	0.760	——	——	——
	B	1.0254	6.3	96.73	6.81	6.084	0.722	——	——	——
	C	1.0215	6.3	96.38	5.77	4.834	0.736	——	——	——
4	A	1.0284	6.3	94.23	8.60	7.768	0.840	0.804	0.481	0.323
	B	1.0274	6.4	94.66	8.08	7.168	0.916	0.729	0.433	0.296
	C	1.0242	6.4	95.88	6.54	5.802	0.738	0.558	0.320	0.238
5	A	1.0285	6.3	93.57	9.28	8.484	0.800	——	——	——
	B	1.0294	6.4	93.60	9.34	8.500	0.836	——	——	——
	C	1.0244	6.4	95.01	7.43	6.712	0.716	——	——	——
When the worms began spinning	A	1.0311	6.4	93.44	9.67	8.860	0.806	1.037	0.747	0.290
	B	1.0276	6.4	92.76	10.00	9.242	0.758	0.801	0.528	0.273
	C	1.0276	6.4	93.25	9.51	8.752	0.750	0.756	0.531	0.225

Mg. in 100 c.c.

		Total phosphate	Organic phosphate	Inorganic phosphate	Chloride	Calsium	Magnesium	Potassium
0		40.1	42.4	6.4	28.3	54.9	155.2	254.0
2	A	85.4	76.5	8.9	28.8	54.8	169.4	149.5
	B	58.4	50.2	8.2	31.1	62.3	188.5	204.5
	C	46.6	41.8	4.8	34.2	66.6	200.3	257.5
3	A	116.2	——	——	——	56.9	133.7	——
	B	76.9	——	——	——	62.2	186.8	——
	C	55.2	——	——	——	67.7	189.8	——
4	A	152.5	141.2	11.3	27.7	57.6	158.5	214.5
	B	112.8	105.0	7.8	31.5	62.4	177.5	229.0
	C	68.9	63.7	5.2	32.7	64.7	191.4	182.0
5	A	147.7	——	——	——	55.7	177.7	——
	B	144.1	——	——	——	61.8	193.1	——
	C	85.6	——	——	——	67.2	178.3	——
When the worms began spinning	A	88.8	82.6	6.2	39.1	70.5	204.3	210.0
	B	80.1	74.3	5.8	41.1	86.1	207.7	186.4
	C	77.3	73.2	4.1	40.5	81.4	195.5	226.0

Group A, which was fed with the youngest leaves, gave always the highest value in specific gravity, in percentage of dry and organic matter, total and protein nitrogen, and total and inorganic phosphate. And group C showed the lowest in them, while B was intermediate. But that was not the case in the percentage of other inorganic matter. In the earlier period of the experiment, group A gave the lowest value in the percentage of inorganic matter, but in the later period the highest value. On the contrary, the changes in group C are the reverse of those in group A, and group B always shows the mean results between A and C. The concentration of calsium, magnesium and chlorides in the blood show a little variation, and was slightly higher in the case of C than of B and A.

Hydrogen ion concentrations are nearly the same in all three cases, but slightly higher in the case of A.

Among these constituents studied, total and protein nitrogen, and total and inorganic phosphates show a great variation. These relations are as similar in the case of the female and the male.

Summary and Conclusion.

(1) Changes in consentration of the blood constituents of the silkworms fed with the mulberry leaves of different stages of development were investigated.

(2) Among several constituents in the blood investigated, concentration of total and protein nitrogen, and total and inorganic phosphates show a great variation according to the difference of mulberry leaves fed. These constituents show high percentage in the blood of the silkworms fed with the young mulberry leaves which are rich in these constituents.

(3) The concentration of calsium, magnesium and chlorides in the blood show a little variation, and are a little higher in the blood of the silkworms fed with the mulberry leaves in full growth, which are very rich in these constitutions.

(4) Most of the constituents of blood vary generally in concentration according to these of the mulberry leaves fed.

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THE CHEMICAL RESEARCHES OF THE "LUN-YUEH" COCOON SILK FROM CANTON.

By

R. Inouye and Chên Chiu Ju.

(Received 23 rd , 1929)

I. General Composition.

About 2.5 killograms cocoons of the polyvoltine species, "Lun–Yueh", which had been raised at the Agricultural College of Canton in Southern China, were obtained for the chemical researches. The cocoons were opened, and the pupae, skins, and other impurities were taken out; thus the total weight of the pure cocoon silk was 360 grams, from which 30 grams were taken for the general analysis. The remained 330 grams of the cocoon silk were steamed in an autoclave under one atmospheric pressure for one hour, and then filtered. The same operation was repeated until the filtrate gave no biuret reaction. The filtrates and washings were united, and evaporated to a small volume, and then sericin was precipitated by 95% alcohol. The sericin thus obtained, was treated with alcohol, and then ether for purification. The cocoon silk, set free from sericin, was 203.9 grams by weight, from which we obtained the following results by the general analysis.

	In 100 parts of the air dry cocoon silk.
Water.	10.65
Dry matter.	89.35
	In 100 parts of the dry matter.
Ash.	1.00
Matter soluble in ether.	2.80
Matter soluble in alcohol.	2.75
Total nitrogen.	17.36
Fibroin.	70.21
Sericin.	21.09

The elementary composition of fibroin and sericin was as follows:

	Fibroin.	Sericin.
Carbon	48.18%	43.68%
Hydrogen	6.20	6.51
Nitrogen	17.38	16.32
Oxygen	27.73	31.99

Various forms of nitrogen in fibroin and sericin.

	Fibroin.	Sericin.
Total nitrogen.	17.62%	16.32%
Nitrogen soluble in con HCl.	17.50	16.23
Nitrogen insoluble in con. HCl.	0.12	0.09
Nitrogen precipitated by phosphotungstic acid among the nitrogens dissolved in con. HCl.	0.89	3.60
Nitrogen not precipitated by phosphotugstic acid among the same nitrogens.	16.28	11.61
Ammonia nitrogen.	0.33	1.03
Amino nitrogen.	14.75	12.36
Non-amino-nitrogen.	2.42	2.84
Arginine nitrogen.	0.56	1.92
Lysine nitrogen.	0.17	0.84
Histidine nitrogen.	0.16	1.33
The amino-nitrogen in the nitrogen precipitated by phosphotungstic acid.	0.36	1.27
Non amino-nitrogen in the nitrogen precipitated by phosphotungstic acid.	0.53	2.33

II. The Amino-acid Composition of the Fibroin separated.

197.3 grams of the dry fibroin were digested with 502 c.c. of 25% sulphuric acid for 16 hours under a reverted cooler, and then filtered. The residue was repeatedly washed with water, until the last washing gave no reaction of sulphuric acid. The hydrolysate and washings were united, from which the sulphuric acid was exactly removed by additing concentrated solution of baryta. The barium sulphate thus produced, was filtered, and several times extracted with boiling water to remove the amino acids contained in the precipitate. The filtrate and extracts were mixed together and evaporated, until tyrosine crystallized out. The filtrate of tyrosine was further evaporated in order to make tyrosine still remaining, to separate out. The operation was repeated until no more tyrosine could be obtained by crystallisation, and the small quantity of tyrosine still remained in the mother liquor, was determined by the volumetric method.

The filtrate of tyrosine was again evaporated under a reduced pressure into a syrup, which was repeatedly treated with absolute alcohol to remove water as quiet as possible. Then the syrup was dissolved in absolute alcohol by passing dry hydrochloric acid gas, and then glycocoll was made to crystallize out as ethyl ester hydrochloride. From the filtrate of it were the

esters of the other amino acids isolated by the ester method of E. Fischer.

Three fractions were made on distillating of the esters of the amino acids, and the yields of the amino acid esters and amino acids were as follows:

Fractions	Heating equipment	Time	Temperature		Pressure	Amino-acid esters	Amino-acids
			outside	inside			
I	Water-bath	60 m.	60°C	45°C	16 mm.	23.00 g.	6.91 g.
II	Water-bath	60	100	63	12	45.30	32.38
III	Oil-bath	40	180	70	5	14.10	6.67
Distillation residue						23.65	2.50

From the fractions thus obtained, proline was extracted with alcohol, after hydrolised and evaporated, and phenylalanine directly by ether. Other amino acids were isotated by fractional crystallization after hydrolysed. Finally the amino acids obtained, were analysed one by one and identified.

The results are summarized up as follows:

Amino-acids.	In 172.9 grams of dry fibroin.	In 100 grams of dry fibroin.
Glycocoll.	50.20 g.	29.03 g.
Alanine.	33.24	19.23
Leucine.	4.98	2.88
Aspartic acid.	1.05	0.61
Glutamic acid.	3.03	1.75
Serine.	2.61	1.51
Phenylalanine.	1.67	0.97
Tyrosine.	15.47	8.94
Proline.	1.06	0.61
Total.	113.31	65.73

From the above results and "Lun-Yueh" silk is chiefly composed with glycocoll, alanine and tyrosine as the other silks are. When compared the results to those, which have been investigated, this silk is similar to the Bengal silk (E. Abderhalden and J. Sington, Zeitschr. f. physiol. Chemie, **61**, 259, 1909). The Lun-Yueh silkworm being the product of Canton and polyvoltine species, is supposed to be similar in the other properties to the Bengal silk besides the amino acid composition.

STUDIES ON THE CASTOR-BEAN LIPASE IV.

INFLUENCES OF MANGANESE SULFATE, MAGNESIUM SULFATE AND ALANINE UPON THE ENZYME ACTION

By

Etsuo Takamiya.

(Received May 24 th., 1929)

Many investigators have hitherto investigated the influences of neutral

salts upon the castor bean lipase but this problem is not yet solved because of their disagreement in experimental results. Therefore, the author investigated with a view to securing accurate data on this problem, and reports here on the influences of manganese sulfate, magnesium sulfate and alanine, which are the most interesting among the neutral salts, upon the enzyme action. The disagreement in the results of the various investigators on this problem should probably be ascribed to the differences or incompleteness of their experimental methods. Hence their methods were examined in detail.

From the fact[1] that manganese salts, magnesium salts and amino acids in the alcoholic solution react as acid against the indicator, phenolphthalein, the author titrated, in the alcoholic solution, the fatty acid produced by the enzymic hydrolysis, using rosolic acid in place of phenolphthalein. Against rosolic acid these substances react neutrally in the alcoholic solution and the acid is suitable for the titration of weak acids as well as phenolphthalein. The two enzyme preparations—raw and highly purified—were employed in this study.

The results showed that manganese sulfate and magnesium sulfate have almost no influence but alanine retards somewhat the enzyme action.

(1) E. Takamiya: J. Agri. Chem. Soc. Japan, **2**, 815, 1121, 1927.

ON THE SYNTHESIS OF VITAMIN BY ASPERGILLUS ORYZAE.

By

Dr. E. Takahashi and H. Lim.

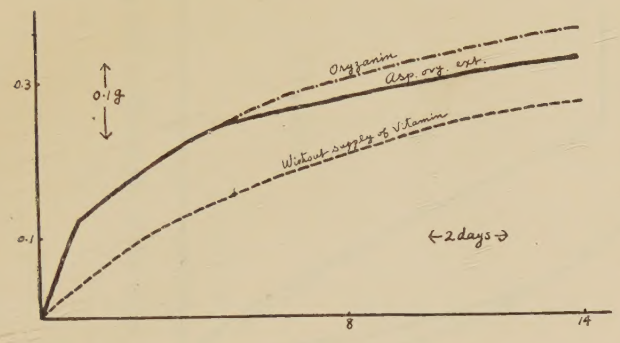
(Received May 27 th , 1929)

We have reported in the former paper on the presence of Vitamin B in Japanese Sake-cake. As to the origin of the Vitamin appeares to have any relation to the Aspergillus oryzae which is added by the fermentation of the wine. So we started to investigate the Vitamin forming power of the fungus whether it really synthesizes the substance or not.

First of all, we prepared a modified pfeffer solution, exactly free from Vitamins and the like substances. After it was ascertained by using Streptococcus pyogenes haemolyticus and Diplococcus pneumoniae (Types I) which are known not to grow under the lack of Vitamin in their media, Aspergillus

oryzae was cultured, at intervals of 2–3 days, up to 65 generations so long for 6 months. Then, the Vitamins even though be transfered from former medium and stock fungus must go gradually decreased by these procedure so faint as to be considered quite negligible. It is noteworthy, Aspergillus oryzae, through these repeated culture could thrive ever as beginning.

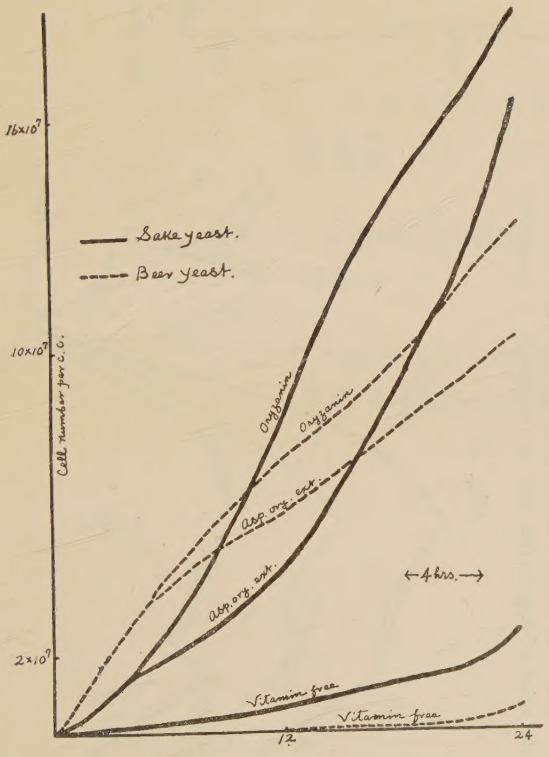
Fig. 1.



The effect of Asp. oryzae ext. on the growth of Asp. oryzae.

Now so long brought up fungus was transplanted in the large quantities of Vitamin free solution above mentioned, after 3 weeks the fungus was gathered, refined with ether and dried. With the Vitamin B extract prepared from the dried fungus growth test for Aspergillus oryzae, yeasts, Streptococcus pyogenes haemolyticus, Diplococcus pneumoniae (Types I) as well as for albino rats and chickens were executed.

Fig. 2.



The effect of Asp. ory. ext. on the growth of yeasts.

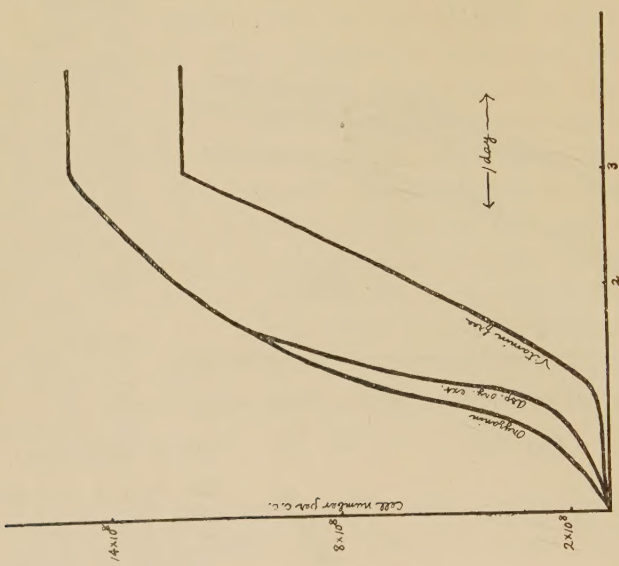
In every case, in the media added with the extract, Aspergillus oryzae and yeasts showed more favorable growth than that of containing no above preparation. In later solution Streptococcus pyogenes haemolyticus and Diplococcus pneumoniae (Types I) could not live at all. Animals also grew healthy only on the diet containing the fungus extract without suffering from polyneuritis.

From these results we may conclude that Aspergillus oryzae can syn-

thesize the Vitamin B, antineuritic and growth promoting factors as well.

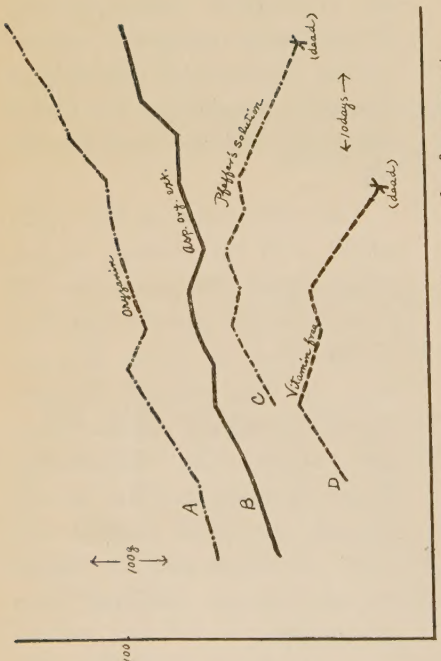
The resultant curves are as follows :–

Fig. 3.



The effect of Asp. oryzae ext. on the growth of Sake-yeast.

Fig. 4.



The effect of Asp. oryzae ext. on the growth of young rats.

Fig. 5.

The curative effect of Asp. oryzae extract upon chicken polyneuritis.

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